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### (54) Amplification of long nucleic acid sequences by PCR

(57) Methods and reagents are provided for the amplification of nucleic acid sequences, e.g. DNA sequences, longer than 10 kilobases by the polymerase chain reaction (PCR). The methods use compositions consisting of a primary thermostable DNA polymerase from *Thermus thermophilus* combined with a lesser amount of a secondary thermostable DNA polymerase possessing a 3'-to-5' exonuclease activity from *Thermococcus litoralis*, *Pyrococcus* species GB-D or *Thermotoga maritima*. The DNA polymerase compositions, when used with the disclosed reaction buffer, enable amplifications of DNA sequences up to at least 42.2 kilobases in length.

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## EUROPEAN SEARCH REPORT

Application Number  
EP 95 10 2141

DOCUMENTS CONSIDERED TO BE RELEVANT			CLASSIFICATION OF THE APPLICATION (Int.Cl.)						
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim							
Y	US-A-5 001 050 (L. BLANCO ET AL.) * column 11, line 20 - line 45 * * column 4, line 13 - line 57 * * column 2, line 60 - column 3, line 12 * * column 2, line 8 - line 28 * ---	1	C12Q1/68 C12Q1/48 C12N9/12						
A		3,4							
Y	PCR METHODS & APPLICATIONS, vol. 3, no. 2, February 1993 COLD SPRING HARBOR US, pages 115-119, L.D. OHLER ET AL. 'Use of a sensitive fluorescent intercalating dye to detect PCR products of low copy number and high molecular weight.' * page 119, column 1, line 17 - line 23 * ---	1							
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Y	NUCLEIC ACIDS RESEARCH, vol. 18, no. 4, 1990 OXFORD GB, pages 999-1005, S KWOK ET AL. 'Effects of primer-template mismatches on the polymerase chain reaction: human immunodeficiency virus type 1 model studies' * the whole document *	1	C12Q C12N						
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<p>The present search report has been drawn up for all claims</p> <table border="1" style="width: 100%; border-collapse: collapse;"> <tr> <td style="width: 33%;">Place of search</td> <td style="width: 33%;">Date of completion of the search</td> <td style="width: 34%;">Examiner</td> </tr> <tr> <td>BERLIN</td> <td>19 December 1995</td> <td>De Kok, A</td> </tr> </table>				Place of search	Date of completion of the search	Examiner	BERLIN	19 December 1995	De Kok, A
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CATEGORY OF CITED DOCUMENTS		<p>T : theory or principle underlying the invention      E : earlier patent document, but published on, or      after the filing date      D : document cited in the application      L : document cited for other reasons      &amp; : member of the same patent family, corresponding      document</p>							
<p>X : particularly relevant if taken alone      Y : particularly relevant if combined with another      document of the same category      A : technological background      O : non-written disclosure      P : intermediate document</p>									



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D, A	PCR METHODS & APPLICATIONS, vol. 2, no. 1, August 1992 COLD SPRING HARBOR US, pages 51-59, L.D. OHLER ET AL. 'Optimization of long-distance PCR using a transposon-based model system' * whole document, esp. page 54, column 3 * ---	1		
A	C.R. NEWTON ET AL. (EDS.) 'PCR' 1994, BIOS SCIENTIFIC PUBLISHERS, OXFORD GB * page 12 - page 16 * ---	1, 2		
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Y	BIOTECHNIQUES, vol. 10, no. 1, January 1991 NATICK US, pages 58-59, D. POMP ET AL. 'Organic solvents as facilitators of polymerase chain reaction' * the whole document * ---	5		
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